

What Is Claimed Is:

1. A Vector Donor DNA molecule comprising a first DNA segment and a second DNA segment, said first or second DNA segment containing at least one Selectable marker, wherein the first and second segments are separated either by, (i) in a circular Vector Donor, a first and a second recombination site, or (ii) in a linear Vector Donor, at least a first recombination site, wherein each pair of flanking recombination sites are engineered and do not recombine with each other.

2. A Vector Donor DNA molecule according to claim 1, wherein the Selectable marker is at least one DNA segment selected from the group consisting of:

- (i) a DNA segment that encodes a product that provides resistance against otherwise toxic compounds;
- (ii) a DNA segment that encodes a product that is otherwise lacking in the recipient cell;
- (iii) a DNA segment that encodes a product that suppresses the activity of a gene product;
- (iv) a DNA segment that encodes a product that can be readily identified;
- (v) a DNA segment that encodes a product that is detrimental to cell survival and/or function;
- (vi) a DNA segment that inhibits the activity of any of the DNA segments of (i)-(v) above;
- (vii) a DNA segment that binds a product that modifies a substrate;
- (viii) a DNA segment that provides for isolation of a desired molecule; and
- (ix) a DNA segment that encodes a specific nucleotide sequence which can be otherwise non-functional; and

- (x) a DNA segment that, when absent, directly or indirectly confers sensitivity to particular compounds.

3. A Vector Donor DNA according to claim 2, wherein said Selectable marker is at least one selected from the group consisting of an antibiotic resistance gene, a tRNA gene, an auxotrophic marker, a toxic gene, a phenotypic marker, an antisense oligonucleotide; a restriction endonuclease; a restriction endonuclease cleavage site, an enzyme cleavage site, a protein binding site; and a sequence complementary PCR primer.

4. A Vector Donor DNA molecule according to claim 1, wherein said Selectable marker comprises at least one inactive fragment of a Selectable marker, wherein the inactive fragment is capable of reconstituting a functional Selectable marker when recombined across said first or second recombination site with a further DNA segment comprising another inactive fragment of the Selectable marker.

5. An Insert Donor DNA molecule, comprising a desired DNA segment flanked by a first recombination site and a second recombination site, wherein the first and second recombination sites are engineered and do not recombine with each other.

6. An Insert Donor DNA molecule according to claim 5, wherein said desired DNA segment codes for at least one selected from the group consisting of a cloning site, a restriction site, a promoter, an operon, an origin of replication, a functional DNA, an antisense RNA, a PCR fragment, a protein or a protein fragment.

7. A kit comprising a container being compartmentalized to receive in close confinement therein at one compartment, wherein a first compartment contains a Vector Donor DNA molecule comprising a first DNA segment and a second DNA segment, said first or second DNA segment containing at least one

Selectable marker, wherein the first and second segments are flanked either by, (i) in a circular Vector Donor, a first and a second recombination site, or (ii) in a linear Vector Donor, a first recombination site, wherein each pair of flanking recombination sites are engineered and do not recombine with each other.

5 8. A kit according to claim 7, further comprising a second compartment containing an Insert Donor DNA molecule comprising a desired DNA segment flanked by a first recombination site and a second recombination site, wherein the first and second recombination sites are engineered and do not recombine with each other.

10 9. A kit according to claim 7, further comprising an additional compartment containing at least one recombination protein capable of recombining a DNA segment comprising at least one of said recombination sites.

15 10. A nucleic acid molecule, comprising at least one DNA segment having at least two recombination sites flanking a Selectable marker or a desired DNA segment, wherein at least one of said recombination sites comprises a core region having at least one engineered mutation that enhances recombination *in vitro* in the formation of a Cointegrate DNA or a Product DNA.

20 11. A nucleic acid molecule according to claim 10, wherein said mutation confers at least one enhancement of said recombination, said enhancement selected from the group consisting of substantially (i) favoring excisive recombination; (ii) favoring integrative recombination; (iii) relieving the requirement for host factors; (iv) increasing the efficiency of said Cointegrate DNA or Product DNA formation; (v) increasing the specificity of said Cointegrate DNA or Product DNA formation; and contributes desirable attributes to the
25 Product DNA.

12. A nucleic acid molecule according to claim 10, wherein said recombination site is derived from at least one recombination site selected from the group consisting of attB, attP, attL and attR.

13. A nucleic acid molecule according to claim 11, wherein said att site is selected from the groups consisting of att1, att2 and att3.

14. A nucleic acid according to claim 10, wherein said core region comprises a DNA sequence selected from the group consisting of:

- (a) RKYCWGCTTTYKTRTACNAASTSGB (m-att) (SEQ ID NO:1);
- (b) AGCCWGCTTTYKTRTACNAACTSGB (m-attB) (SEQ ID NO:2);
- (c) GTTCAGCTTTCKTRTACNAACTSGB (m-attR) (SEQ ID NO:3);
- (d) AGCCWGCTTTCKTRTACNAAAGTSGB (m-attL) (SEQ ID NO:4);
- (e) GTTCAGCTTTYKTRTACNAAAGTSGB(m-attP1) (SEQ ID NO:5);

and a corresponding or complementary DNA or RNA sequence, wherein R=A or G; K=G or T/U; Y=C or T/U; W=A or T/U; N=A or C or G or T/U; S=C or G; and B=C or G or T/U.

15. A nucleic acid according to claim 14, wherein said core region comprises a DNA sequence selected from the group consisting of:

- (a) AGCCTGCTTTTTGTACAAACTTGT (attB1) (SEQ ID NO:6);
- (b) AGCCTGCTTCTTGTACAAACTTGT (attB2) (SEQ ID NO:7);
- (c) ACCCAGCTTCTTGTACAAACTTGT (attB3) (SEQ ID NO:8);
- (d) GTTCAGCTTTTTGTACAAACTTGT (attR1) (SEQ ID NO:9);
- (e) GTTCAGCTTCTTGTACAAACTTGT (attR2) (SEQ ID NO:10);
- (f) GTTCAGCTTCTTGTACAAAGTTGG (attR3) (SEQ ID NO:11);

(g) AGCCTGCTTTTTTGTACAAAGTTGG (attL1) (SEQ ID NO:12);

(h) AGCCTGCTTCTTGTACAAAGTTGG (attL2) (SEQ ID NO:13);

(i) ACCCAGCTTCTTGTACAAAGTTGG (attL3) (SEQ ID NO:14);

(j) GTTCAGCTTTTTTGTACAAAGTTGG (attP1) (SEQ ID NO:15);

(k) GTTCAGCTTCTTGTACAAAGTTGG (attP2,P3) (SEQ ID NO:16);

and a corresponding or complementary DNA or RNA sequence.

16. A method for making a nucleic acid molecule, comprising providing a nucleic acid molecule having at least one engineered recombination site comprising at least one DNA sequence having at least 90% homology to at least one of SEQ ID NOS:1-16.

17. A nucleic acid molecule provided by a method according to claim 10.

18. A composition, comprising a nucleic acid molecule according to claim 10.

19. A kit, comprising a container being compartmentalized to receive in close confinement therein at least one compartment, wherein a first compartment contains a composition according to claim 18.

20. A kit according to claim 19, further comprising a second compartment having at least one recombination protein that recognizes said recombination site.

21. A kit comprising a container being compartmentalized to receive in close confinement therein at least one recombination protein in isolated form, useful for a method according to claim 22.

5 22. A method of making a Cointegrate DNA molecule, comprising combining *in vitro*:

- 10 (i) an Insert Donor DNA molecule, comprising a desired DNA segment flanked by a first recombination site and a second recombination site, wherein the first and second recombination sites do not recombine with each other;
- (ii) a Vector Donor DNA molecule containing a third recombination site and a fourth recombination site, wherein the third and fourth recombination sites do not recombine with each other; and
- 15 (iii) at least one site specific recombination protein capable of recombining said first and third recombinational sites said second and fourth recombinational sites;

thereby allowing recombination to occur, so as to produce a Cointegrate DNA molecule comprising said first and third or said second and fourth recombination sites.

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20 23. A method according to claim 22, wherein a Product DNA molecule is produced from said Cointegrate DNA by recombining at least one of (i) said first and third, or (ii) said second and fourth, recombination sites, said Product DNA comprising said desired DNA segment.

25 24. A method according to claim 23, wherein said method also produces a Byproduct DNA molecule.

25. A method according to claim 23, further comprising selecting for the Product DNA molecule.

26. A method as claimed in claim 22, wherein the Vector Donor DNA molecule comprises a vector segment flanked by said third and the fourth recombination sites.

27. A method as claimed in claim 22, wherein the Vector Donor DNA molecule further comprises (a) a toxic gene and (b) a Selectable marker, wherein the toxic gene and the Selectable marker are on different DNA segments, the DNA segments being separated either by (i) in a circular DNA molecule, two recombination sites, or (ii) in a linear DNA molecule, one recombination site.

28. A method as claimed in claim 22, wherein the Vector Donor DNA molecule further comprises (a) a repression cassette and (b) a Selectable marker, repressed by the repressor of the repression cassette, and wherein the Selectable marker and the repression cassette are on different DNA segments, the DNA segments being separated either by, (i) in a circular DNA molecule, two recombination sites, or (ii) in a linear DNA molecule, one recombination site.

29. A method as claimed in claim 22, wherein at least one of the Insert Donor DNA molecule and the Vector Donor DNA molecule is a circular DNA molecule.

30. A method as claimed in claim 22, wherein at least one of the Insert Donor DNA molecule and the Vector Donor DNA molecule is a linear DNA molecule.

31. A method as claimed in claim 22, wherein the selecting step is carried out *in vitro* or *in vivo*.

32. A method as claimed in claim 22, wherein said recombination protein comprises at least a first recombination protein and a second recombination protein, said second recombination protein being different from said first recombination protein.

33. A method as claimed in claim 22, wherein said recombination protein is Int.

34. A method as claimed in claim 22, wherein the at least one recombination protein is selected from (i) Int and IHF and (ii) Int, Xis, and IHF.

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